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(54) A method of substantially continuously monitoring the level of a bioactive material.

(57) A method of substantially continuously monitoring the level of a bioactive material transferred through skin or mucosal membrane comprises placing separate electrodes on the skin or mucosal membrane, applying current to the skin or mucosal membrane, substantially continuously transferring the bioactive material through the skin or mucosal membrane and collecting the same and monitoring the collected bioactive material.

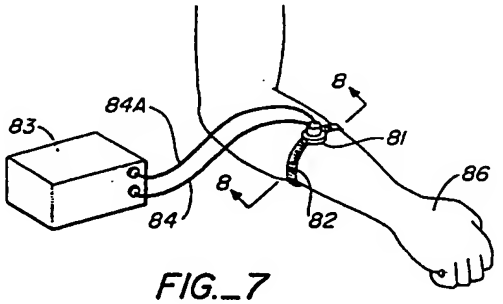

FIG._7

EP 0 673 622 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	US-A-3 794 910 (D. NINKE ET AL.) * the whole document *	1,3,5,7	A61B5/00 A61N1/30 A61B5/14
X	MEDICAL AND BIOLOGICAL ENGINEERING AND COMPUTING, vol. 16, no. 2, March 1978 STEVENAGE GB, pages 126-134, S.Y. SHAYA ET AL. 'Percutaneous electrophoresis of amino acids and urea' * the whole document *	1,3,7	
X	EP-A-0 060 451 (MEDTRONIC, INC.) * page 2, line 3 - line 8 * * page 16, line 1 - line 7; claim 8; figures *	1,3,4,7	
A,D	ARCHIVES OF PATHOLOGY & LABORATORY MEDICINE, vol. 111, no. 7, July 1987 CHICAGO, US, pages 633-636, K.W. HUNTER ET AL. 'Technological Advances in Bedside Monitoring: Biosensors' * the whole document *	1,3,5,7	
A	GB-A-2 033 575 (P. ROLFE) * the whole document *	1-7	
A	US-A-4 365 637 (W.C. JOHNSON) * the whole document *	1-7	
A	US-A-4 195 641 (W.T. JOINES ET AL.) * the whole document *	1-7	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A61B
A61N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	11 September 1995	Ferrigno, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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T : theory or principle underlying the invention
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& : member of the same patent family, corresponding document

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EP 0 673 622 A2

BACKGROUND OF INVENTION

Origin of the Invention

The present invention is a divisional application of EP 89 300 782.3/0 326 398 filed January 27, 1989.

Field of the Invention

The present invention relates to a device and to an in vitro method for modeling the iontophoretic sampling or delivery of substances through a membrane, such as the excised skin of a mammal. In another aspect, the present invention relates to a device and to a method for the iontophoretic delivery or sampling of substances through the intact skin of a living mammal. Specifically, the apparatus is a device which placed on the same side of intact skin has a positive electrode, a negative electrode and an electrically insulating material separating the electrodes. In still another aspect the present invention relates to an iontophoretic method of continuously monitoring the levels of bioactive materials in a subject and using a feedback mechanism to maintain effective levels.

DESCRIPTION OF RELATED ART

Sampling-In-Vitro

C. C. Peck et al. in Pharmacology Skin, Vol. 1, pp. 201-208 published by Karger, Basel 1987, discloses a method to determine in vitro the outward transdermal migration of theophylline using a passive transdermal collection system (TCS). The use of electrical enhancement of the migration is not disclosed.

R. R. Burnette et al. in the Journal of Pharmaceutical Sciences, Vol. 75, No. 8, pp. 738-743, published in August 1986 using the standard diffusion cell discloses a comparison of the iontophoretic and passive in vitro transport of thyrotropin releasing hormone (TRH) across excised nude mouse skin. The results indicate that both charged and uncharged TRH fluxes across the excised tissue were greater than those obtained by passive diffusion alone.

In the standard (state of the art) arrangement for in vitro iontophoretic studies (See Figure 6), the two halves of a diffusion cell are placed horizontally side by side so that the skin is located vertically between them, with its epidermal side facing one half and its inner side facing the other. The bioactive preparation and the active electrode are put in the "epidermal" half of the cell, and the other side of the cell contains the passive electrode in a conductive fluid.

This side-by-side arrangement has several drawbacks and limitations. Since the passive electrode is, in effect, placed "inside" the skin, this configuration is not a good model of the in vivo case. The factors that influence such a non-physiological situation may not be those that are important in the clinical case. In addition, there are questions that cannot be investigated with a side-by-side configuration, such as the possibility of horizontal transport (i.e. within skin layers rather than vertically through the skin) and whether an iontophoretically driven drug is "pulled" back out of the skin by the passive electrode.

A state of the art iontophoretic drug delivery system, the Phoresor, is sold by Motion Control, Inc., 1290 West 2320 South, Suite A, Salt Lake City, Utah 84119.

Delivery-In-Vitro

In modeling studies, iontophoresis is useful to examine chemical transport of charged materials through a membrane, such as an excised skin sample. For instance, N.H. Bellantone, et al. in the International Journal of Pharmaceutics, Vol. 30, pp. 63-72, published in 1986, disclose a standard state-of-the-art side-by-side diffusion cell design and electrode configuration for various systems utilized for iontophoresis of benzoic acid (as a model compound) (see Figure 6). A number of limitations exist with the side-by-side cell design as is discussed further herein.

Delivery-In-Vivo

Iontophoresis is the electrically enhanced transport of charged substances usually bioactive materials. The procedure is a known means of transdermal drug delivery. For instance, in U.S. Patent No. 4,141,359, by S.C. Jacobsen et al., which is incorporated herein by reference, disclose an improved iontophoresis device for the topical administration of ionic drugs or chemicals through epidermal tissue without mechani-

cal penetration. Th positive and negativ electrodes ar attached to the skin at s parate locations. The ionic form of th drug is added to the appropriate electrode and is conducted into and through the epidermal tissu by means of direct current from a power source. A number of problems exist in this type of delivery, where the lectrodes ar separate.

Sampling-In-Vivo

There is a well-recognized and important need to sample and quantify bioactive substances in the body (typically, the blood). For example, it may be crucial to monitor the presence of a key endogenous biochemical for the purpose a disease diagnosis, or it may be essential to follow, and hence, optimize, the blood level of an administered drug during a chemotherapeutic regimen. Usually, the desired determination is achieved by analysis of a blood sample which is withdrawn invasively via an injected needle into a collection tube.

The passive transdermal collection of theobromine in vivo is also disclosed by C.C. Peck, et al. 1987, supra. No electrical current enhancement of the migration is disclosed.

No literature was found which describes a substantially noninvasive procedure for biomaterial sampling of the systemic circulation. It will require a unique application of iontophoresis to "extract" systemically circulating molecules into a collection device positioned on the skin or mucosal membrane surface. The present invention does not involve puncture of the skin nor of any blood vessel.

Biosensing-In-Vivo

There exists a need to continuously or non-continuously monitor certain key biochemical parameters in hospitalized patients, and a need for a new class of medical devices to obtain real-time, on-line quantitation. A biosensor is a microelectronic device that utilizes a bioactive molecule as the sensing signal-transducing element.

K.W. Hunter, Jr., in Archives of Pathological Laboratory Medicine, Vol. III, pp. 633-636, published in July 1987, discloses in a general manner the range of devices and the physical properties which are examined. Hunter also includes a general diagram for a transdermal dosimeter. This reference does not provide needed additional specific information to create an operating biosensing-feedback-drug delivery system.

C.C. Peck et al. in the Journal of Pharmacokinetics and Biopharmaceutics, Vol. 9, No. 1, pp. 41-58, published in 1981, discusses the use of continuous transepidermal drug collection (CTDC) in assessing drug in-take and pharmacokinetics. It was concluded that when back transfer is minimized, CTDC may be a useful tool to access the amount of drug exposure, etc., but offers little advantage over discrete sampling of other body fluids in the study of other aspects of drug disposition kinetics.

U.S. Patents of interest include: 4,329,999; 4,585,652; 4,708,716; 4,689,039; 4,702,732; 4,693,711; 4,717,378; 4,756,314; 4,699,146; 4,700,710; 4,706,680; 4,713,050; 4,721,111; 4,602,909; 4,595,011; 4,722,354; 4,722,726; 4,727,881; 4,731,049; 4,744,787; 4,747,819; 4,767,401.

Y. B. Bannon, European Patent Application Publication No. 252,732 (January 13, 1988) to a transdermal drug delivery system is of general interest.

References of interest include:

W. Scharamm, et al., "The Commericalization of Biosensors," MD&GI, pp. 52-57, publised in November, 1987.

A.F. Turner, et al., "Diabetes Mellitus: Biosensors for Research and Management," Biosensors, Vol. 1, pp. 85-115, published by Elsevier Applied Science Publishers, Ltd., England, 1985.

Y. Ikarlyaman, et al., Proc. Electrochem. Soc., 1987, 87-9 (Proc. Symp. Chem. Sens.) 378. CA 107-(22); 207350n.

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H. Wollenberger, et al., K. Anal. Lett., 1987, 20(5), 857, CA 107(9); 73551.

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I. Hanning, et al., Anal. Lett., 1988 19(3-4) 461, CA 105(6); 48993q.

M. Shirchirl, et al., Diabetes Care, 1986, 9(3), 298. CA 105(5): 38426t.

S.J. Churchouse, et al., Anal. Proc., (London) 1986, 2395), 146 CA 105(3) 21117v.

D.A. Gough, et al., Anal. Chem., 1985, 67(12), 2351. CA 103(15); 11925a.

C. Loo, t al., Chem. Eng. Sci., 1985, 40(5), 873 CA 103(5); 34337a.

All of the r ferences and patents cited herein ar incorporated by reference in th ir entir ty.

It is desirable to have a device and a methodology to sampl (or deliver) substances (charged or neutral) from (or to) a membrane (in vitro) or to sample (or deliver) substances (charged or n utral) from (or to) the intact skin (muscosa, etc.) of a living mammal. The present invention accomplishes these objectives.

SUMMARY OF THE INVENTION

The present invention relates to a diffusion cell device for use in the electrically enhanced sampling of a substance from a membrane surface or the delivery of a substance into or through a membrane surface without mechanical penetration comprising

at least two electrically conducting permeable electrode means for contacting the membrane surface, and

means for electrically isolating each electrically conducting electrode means from each other,

wherein said electrode means are disposed in substantially a side-by-side relationship having sides extending and terminating in a substantially common face surface which contacts immediately adjacent portions of the same side of said membrane surface.

In another aspect, the present invention relates to an in vitro device for the removal or delivery of either ionized or unionized substances from a membrane sample without mechanical penetration, which device comprises:

(a) a positive electrode;

(b) a negative electrode, and

(c) electrical insulation between subpart (a) and (b), wherein the positive electrode, and the negative electrode, and electrical insulation are positioned on the same side of the membrane sample.

In another aspect, the present invention relates to a device for the removal of or delivery of ionized substances to a mammal through intact skin or mucosal membrane without mechanical penetration, which device comprises:

(a) a positive electrode,

(b) a negative electrode, and

(c) an electrically insulating material between subpart (a) and (b), wherein the positive electrode, negative electrode and insulating material are physically positioned so that each present a single common surface of the device for contact with the same side of the skin or mucosal membrane of the mammal.

In another aspect, the present invention relates to the use of iontophoresis to determine the level of a uncharged or charged molecule in a living mammal, and with the use of a feedback mechanism, administer appropriate levels of therapeutic substance by any number of available administration routes.

In another aspect the invention relates to the use of iontophoresis to enhance the collection of a charged or neutral substance from a membrane or the skin of a living mammal at one electrode followed by analysis of the concentration of the substance by gas chromatography (GC), mass spectrometry (MS), by high pressure liquid chromatography (HPLC), scintillation counting, and the like.

Brief Description of the Drawings

Figure 1 shows an isometric view of the in vitro diffusion cell configuration for the modeling of the iontophoretic removal or delivery of a charged or neutral substance.

Figure 2 shows the cross sectional view of the modeling diffusion cell of Figure 1 along line 2-2.

Figure 3 shows an exploded cross-sectional view of the diffusion cell of Figure 2.

Figure 4 shows an exploded cross-sectional view of the cell of Figure 2 wherein solid glass is the insulator in the lower reservoir separating the electrodes. Figure 4 also shows the circulating system for the receptor liquid.

Figure 5A to 5H shows a cross-sectional view of a number of configurations for the positive electrode, negative electrode and insulating means therebetween taken along line 5A-5A of Figure 1 for the bottom of the upper portion of the diffusion cell.

Figure 6 shows an isometric view of the state of the art side-by-side iontophoresis cell.

Figure 7 shows an isometric view of an iontophoresis cell as it is used for in vivo delivery of a bioactive molecule to a human patient.

Figure 8 shows a cross-sectional view of the diffusion cell of Figure 7 along line 8-8.

Figures 9A and 9B shows a top and a bottom vi w of an iontophor sis diffusion c ll of Figur 7 or 8.

Figure 10 shows a cross-sectional view of an iontophoresis experiment wher in th electrodes ar separated.

Figure 11 shows the iontophoretic in vitro sampling of clonidin using the diffusion c ll of Figure 1, 2 or 3.

Figure 12 shows th iontophor tic in vitro sampling of theophylline using the diffusion cell of Figure 1, 2 or 3.

Figure 13 shows the separated electrodes connected to a guinea pig in either a delivery mode or a sampling mode for a charged or uncharged substance.

Figure 14 shows the iontophoretic in vitro delivery of morphine using the diffusion cell of Figure 1, 2 or 3.

Figure 15 shows the iontophoretic in vitro delivery of P.S.O.S. (potassium sucrose octasulfate) using the diffusion cell of Figures 1, 2 or 3.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

Definitions

As used herein:

"Diffusion cell" refers to the electrical system for iontrophoresis. The system may include positive electrode, negative electrode and electrical insulation therebetween. The system may also be a positive lead, electrical insulation and ground.

"Mammal" refers to the usual laboratory animals used in experimentation rats, mice, guinea pigs, rabbits, monkeys and the like. The term may also include dogs, cats, cattle, sheep, horses and the like. A preferred mammal is a human being.

"Membrane surface" refers to either a thin membrane such as excised skin, synthetic membranes, mucosal membranes or to the surface and below the intact skin of a mammal, preferably a human being.

A preferred embodiment for sampling or delivery is the combination of materials for the permeable electrodes.

A more preferred embodiment of the electrodes includes a metal wire in combination with a gel which is in contact with the membrane surface.

General Materials and Methods for Sampling or Delivery

Biomaterial or biomaterials delivered or sampled includes anything found in the system of a living mammal such as degradation products, metal ions, peptides, hormone toxins, and the like—neutral species or those which carry or can be made to carry an electrical charge.

In the sampling and in the delivery electrode (which are open), the electrically conducting gel is KENZGELELC available from NITTO Electric Industrial Co., Osaka, Japan.

Voltage is normally between about 0.1 and 15 volts, preferably between about 1 and 10 volts, and especially about 5 volts for both sampling and delivery.

In-Vitro-Sampling

Description of modeling cell: Refer to Figures 1, 2, 3 and 4. The iontophoretic diffusion cell 10 for in vitro model sampling is constructed so that one half of the cell 11 is above the other half 12. The excised skin 13 is interposed horizontally with the epidermal surface 14 interfacing with the upper half of the cell (see Figure 1, 2 or 3). The upper half of the cell 11 is divided by two vertical walls 15A and 15B into three chambers 16A, 16B and 16C. The outer two chambers are therefore separated by an intervening space 16B (the third chamber). The lower half of the cell 12 holds the receptor fluid. The walls 15A and 15B that form the middle chamber 16B in the upper half 11 are continued as walls 15C and 15D into the lower half of the cell 12, but are then joined to form a trough 18 producing a channel 19 which traverses the top of the lower half of the cell 12. Channel 19 may have sampling ports to remove liquid.

Chambers 16A and 16C each independently contain an electrically conducting means or medium 25A (or 25B) selected from a gel, liquid, paste, sponge, foam, emulsion, permeable metal, permeable ceramic or combinations thereof. To complete the electrical circuit, usually metal wires or electrodes 26A and 26B are each placed in the electrically conducting medium as shown in Figure 3. The other similar Figures, e.g. 1, 2, 3 or 4 can be interpreted in the same manner.

When the upper half of the cell 11 is positioned over the lower half 12 so that the upper walls 15A and 15B and lower walls 15C and 15D coincide, the strip of skin 13 betw en the walls is sealed off from the skin in the electrod chambers on both its tipp r and lower sides (Figure 2). Th portions of skin in the electrode

chambers 16A and 16C are thus physically and lectrically isolated from each other so that th flow of current and biomaterial through and within the skin can be investigated. Ports 20A and 20B in the lower half cell 12 allow recept r fluid 17 to b continuously perfused. Ports 21A and 21B allow for constant temperature liquid monitoring of water jacket 21C. During an exp riment, the channel 19 at the top of the lower half of the cell 12 is filled with receptor fluid 17 so that the underside of the skin 13 remains moist. The walls 15C and 15D of the channel also provide mechanical support for the membrane (skin).

The upper half 11 and lower half 12 are combined at their complementary single plane faces, having the sample 13 therebetween, using spring clips 22A and 22B or other fastening means to keep the upper half 11 and lower half 12 of the cell tightly joined together.

In Figure 4, barrier 18A is made of glass to electrically insulate chambers 16A and 16C. Figure 4 also shows the receptor liquid 17 moving in line 20B to a container 29A. The liquid 29B is then pumped using a pump 29 back to the reservoir (or vice versa). Any suitable liquid, saline, blood and the like may be used in both substance sampling or delivery experiments for substances.

Shown in Figures 5A to 5H are several embodiments of the spatial configuration of the diffusion cell 10 of the present invention along line 5A-5A of Figure 1. Figure 5A is a cross-sectional view of the bottom of the upper half of Figure 1 taken across line 5A-5A. Chambers 16A, 16B and 16C and electrical isolating materials 15A and 15B (e.g. glass walls) are shown. In Figure 5B is a cross-sectional view along line 5-5 having chamber 16A and 16C and a single glass wall 15E. Cross-sectional view 5C has square chamber 16A, 16B and 16C with electrically insulating walls 15A and 15B. The cross-sectional view of Figure 5C has square chambers 16A and 16C and a single glass wall 15F. The cross-sectional view in Figure 5E shows a concentric coaxial configuration. The chambers 16AA(+), 16BB (insulating) and 16CC(-) are shown having insulating glass walls 15AA and 15BB and 15EE. The cross-sectional view of Figure 5F also shows a concentric cell having chambers 16AA(+) and 16CC(-) and insulating wall 15CC and 15FF. Figure 5G is an cross-sectional view of electrodes and insulation having two circular electrodes (chambers 16A and 16C) and glass insulating walls 15G and 15H. Figure 5H is a cross-section of a type or concentric cells along line 5A-5A having chambers 16A and 16C with insulating wall 15DD. These configurations appear on the bottom of upper half 11 and the top of lower half 12 and the walls and chamber coincide when the cell is closed as shown in Figure 1.

Of course, the top of upper half 11 is shown having an open top to the chambers 16A to 16C. This top may be closed or covered. In this way, the chambers 16A and 16C containing the electrode wires could be positioned at many angles from the vertical, and the chambers 16A and 16C would retain good electrical contact with the membrane surface.

To maintain a solid electrical contact, a chemical adhesive which is not susceptible to iontophoresis, such as the hypoallergic chemical adhesive available from 3M Company, St. Paul, Minnesota, may be used.

A non-invasive method and device for sampling and monitoring of non-ionic moieties, such as glucose, sucrose and the like, using iontophoresis is described.

Glucose--Medical diagnosis and patient care rely upon sampling and analysis of bioactive substances in the body. Typically, sampling involves analysis of the blood and plasma which implies an invasive, inconvenient, risky (e.g. viruses) and some times limited blood sampling. One of the most important cases where sampling is needed, at least several times a day for life time, is in the case of patients having sugar diabetes. Real time information concerning the glucose levels in the body (e.g. blood) is most important information in the patient's treatment and in many cases--often a question of life and death. A simple non-invasive sampling method using iontophoretic sampling is now described.

In order to show the ability of the method to sample glucose through the skin, in vitro studies are conducted using hairless mouse skin as the skin model and the iontophoretic diffusion cells in Figures 1-13. The results here are applicable to in-vivo sampling from a mammal, particularly a human being. Two self-adhesive gel electrodes (Kenzgelelc, by Nitto Electric Industry Co., Limited, Osaka, Japan) are placed on the same side of a single continuous piece of hairless mouse skin (full thickness-about 0.5 mm) (Skh: hr-1, 8-13 weeks old). Under the skin, radiolabelled (^{14}C-U)-glucose having known concentration in solution in phosphate-buffered saline (0.9% sodium chloride) is perfused, pH of phosphate is about 7.4. Temperature is ambient.

In the first set of experiments, following the assembly of the cell and initiation of the glucose solution perfusion, current is applied (0.5 milliampere) for 2 hours. The voltage may vary from about 1 to 10 volts. Usually, it is about 5 volts. The important parameter to keep constant is the appli d current. The voltage may vary based upon the el ctrical resistanc of the sampling site. Then th g l lectrod s ar disconnected and t st d for radioactivity content by conventional liquid scintillation counting. When the glucose concentration under the skin is changed from 1.07 mg/ml to 0.153 mg/ml (a factor of 0.143), the amount sampled through the skin in 2 hours is changed from 4.9μg to 0.705μg (a factor of 0.144 - se

Table 1). The results demonstrat that for a fixed sampling time under th same electrical conditions, a near-perfect linear correlation between the glucose concentration in the skin and th amount of glucos sampled, is obtained at the (+) electrod . (Electrical conditions in the experiments --same constant direct current (dc) but as iontophoretic delivery may also operat in pulsed curr nt, etc., (the pulsed approach should also perform in a predictable way for sampling).

TABLE 1

GLUCOSE SAMPLE	
Concentration[mg/ml]	Iontophoretic Flux sample(μg/2hr)
A 0.153	0.705 +/- 0.095
B 1.07	4.9 +/- 0.7 (+/- 14%)
Ratio A/B 0.143	0.144 (Found)

I n the second set of experiments, using similar experimental set-up as before, a (^{14}C-U)-glucose solution of 0.34 mg/ml glucose is phosphate buffered saline is perfused, and the gel electrodes are replaced every 30 minutes.

Evaluation of the glucose sampled into the electrode gel shows a repeatable amount of radioactive glucose-0.8μg/0.5 hr. with a standard deviation (S.D.) of ± 23% (see Table 2). Since the skin for each single experiment comes from a different mouse, evaluation of each single diffusion cell (means - samples collected through the same piece of skin), eliminating the first sample (the first 0.5 hr. is slightly higher than the others due to the experimental conditions) was done. The experimental values obtained of 0.79 to 0.74μg are an average of the amount of glucose found in four separate cells.

TABLE 2

GLUCOSE SAMPLING		
Sample	Time (hr)	Sample-glucose (μg)
1	0 - 1/2	0.97 +/- 0.12
2	1/2 - 1	0.79 +/- 0.09
3	1 - 1 1/2	0.76 +/- 0.10
4	1 1/2 - 2	0.75 +/- 0.21
5	2 - 2 1/2	0.74 +/- 0.24
		Average 0.80 +/- 0.19 (+/- 23%)
Concentration of glucose is 0.34 mg/ml Flow rate of glucose solution is 15 ml/hr n = four cells were averaged for each sample		

It is demonstrated that the S.D. for individual diffusion cells, means individual mouse, are within the range of 4 to 9% (except for cell #3) - see Table 3.

TABLE 3

GLUCOSE SAMPLING	
Cell*	Amount Glucose Measured In Gel Electrode per Sample (μg)
1	0.825 +/- 0.041 (+/- 5%)
2	0.963 +/- 0.090 (+/- 9%)
3	0.530 +/- 0.115 (+/- 22%)
4	Cell broken
5	0.727 +/- 0.033 (+/- 4%)
Concentration of glucose is 0.34 mg/ml Flow rate of glucose solution is 15 ml/hr n = Averages of four cells for each of four time periods	

It is demonstrated that glucose is iontophoretically sampled accurately by the present invention. There is a clear correlation between the amount of glucose under the skin, and the amount of glucose that is sampled. The glucose amounts sampled are significant and repeatable and therefore reliable. Since it is known that iontophoretic transport is linearly dependent upon current and duration of current flow these parameters are safely manipulated (within safe limits for current concentration) in order to obtain detectable amounts of glucose in the gel electrode. This method is not limited to transdermal sampling and is possible through mucosal surfaces (e.g., nasal, buccal) where the barrier for non-ionized species transport is much lower, and the concentration of blood vessels is high.

The combination of this sampling procedure with specific glucose biosensor (e.g., J.C. Cooper, E.A.H. Hall, Journal of Biomedical Engineering, Vol. 10, pp. 210-219, published in 1988), or glucose selective electrodes (R.L.Solsky, Analytical Chemistry, Vol. 60, #12, 106R-113R, published in 1988), or in situ analysis (e.g., colorimetric) provides real time glucose information, which references are incorporated herein by reference.

Combination of the above monitoring, which provide real time medical information with delivery device (e.g., insulin pump, iontophoretic insulin delivery device) produces a useful closed-loop, "feed-back" drug delivery system.

Drugs whose levels in a human being are sampled and monitored by the present method include, but are not limited to:

Agent	For
Theophyline treatment	Blood levels for asthama
Fluorouracil methotrexate	Blood levels in cancer chemotherapy
Metal ions K^+, Na^+, Cu, Fe^{+++}, etc.	Examine blood levels
Accidental poisoning	Where invasive blood sampling is to be avoided
Concentration of suspect agent (e.g., drugs of abuse)	No invasive concentration of agent
Hormone levels	Monitor blood levels
Prostaglandin (nasal) steroids (anabolic cancer treatment, male or female hormone adjustment)	Monitor blood levels
Antidepressants amitriptylene •HCl	Sample and monitor blood levels

The present invention is useful in determining metabolic glucose levels in a mammal, preferably a human being. Both hypoglycemic and hyperglycemic conditions are monitored, e.g. from a glucose level in milligrams per milliliter of blood of about 0.1 mg/ml to 5.0 mg/ml. A preferred range for monitoring hypoglycemia is between about 0.3 and 0.7 mg/ml. A preferred range for hyperglycemia (diabetes) between about 1.0 and 5.0 mg/ml. A normal blood glucose level is between about 0.8 and 1.1 mg glucose/ml of blood.

Biosensors for detection at concentration l vels of inter st are commercially available for the analysis of lactate, alcohol, sucros , glactose, uric acid, alpha amylase, choline and L-lycine, and all are indirect amperometric based on oxygen consumption or hydrogen peroxide production. Ther are a few commercially available biosensors which are based on alternative methods of detection. The most important of

these from a commercial point of view is the NAIAD automatic chemical warfare ag nt detector.

The ExacTech (Baxt r Travenol, Deerfield, Illinois) biosensor for glucos is a second generation biosensor of amperometric operation. Oxygen is r placed by an artificial electron mediator, which shuttles the electrons from th biological component to the electrode. Such revolutionary mediators: (1) exhibit ready participation in redox reactions with both the biological component and the electrode; (2) show stability under the required assay conditions; (3) do not tend to participate in side reactions during transfer of electrons, such as the reduction of oxygen; (4) exhibit appropriate redox potentials away from that of other electrochemically active species that may be present in samples; (5) are unaffected by a wide range of pH; are non-toxic, especially for in vivo applications; and (6) are amenable to immobilization.

The ExacTech glucose test is easily performed and a result is obtained within 30 seconds of applying the whole blood sample to a disposable of a pen-sized device. In the currently envisioned improved use of this kind of device, the disposable strip placed in the device is replaced with a material wetted with glucose which has been drawn through the skin iontophoretically (without drawing blood). The sampling matrix is made of polyvinyl chloride, as is the disposable strip, or it may be made of some other material with better characteristics for iontophoretic sampling. The loading of the designated matrix is done as a separate step or, preferably, as part of an assay with simultaneous or concerted sampling and detection of glucose. The matrix for detection may be a disposable strip, as in the current detection system for blood, and used only once, or it may be a material which allows multiple sampling. The latter matrix may remain in place indefinitely or it may only be useful over a set time (number of assays). Preferably, it will be in proper juxtaposition with the electrode to allow the concerted assay. However, it is configured with the device (monitoring system) with respect to sampling and detection, the moistened matrix containing iontophoretically drawn glucose is applied over the accessible electrode area at the free end of the electrode. Here glucose oxidase catalyzes oxidation of the glucose, with the electrons generated being transferred to the underlying electrode via a mediator. The magnitude of the current generated is proportional to the blood glucose concentration in the sample and is displayed in mg/dL on the liquid crystal display built within the monitor. As they become commercially available, other comparably or even more sensitive means of detecting glucose may be substituted for the above described, commercially available blood sampling system.

Delivery-In-Vitro

The in-vitro delivery of a substance to a membrane of the present invention will also utilize Figures 1, 2, 3, 4 and 5. Figure 6 shows for reference the state-of-the-art in vitro delivery system 60. The horizontal electrodes 61A and 61B combine on vertical membrane 62. The receptor liquid 63 originally will not contain any of the substance in liquid 64. When the circuit 65 is completed with power supply 66, the substance in 64 moves into and through membrane 62 and will appear in receptor liquid 63.

The equipment and technique for delivery in the present invention is similar to that described above for the in-vitro sampling. Any conductive material such as metals (platinum, aluminum, etc.), conductive gels (e.g. with sodium chloride solution, etc., conductive solutions (sodium chloride in water, etc.) or any combination of these materials.

The permeable electrodes of the present invention range in size from about 1 μm^2 to 400 cm^2, preferably about 1 mm^2 to 40 cm^2. The current density is about 0.01 $\mu A/cm^2$ to 2 mA/cm^2, preferably 1 $\mu A/cm^2$ to 0.5 mA/cm^2. The electrodes may be attached by straps, adhesive, bands and the like.

The same membranes as described in in vitro sampling are pertinent here, e.g., full thickness or split thickness, skin, mucosal membranes, artificial polymer membranes and the like.

Sampling-In-Vivo

The figures useful in illustrating in vivo sampling are numbers 5, 7, 8, 9A and 9B. The information found above for in vitro sampling can be adapted and applied here.

In Figure 7 is shown one embodiment of the sampling. The exterior of top cell 81 looks very similar to top half 11 but has the shape of top 81. The electrodes appear to be similar or identical chambers 16A, 16B and 16C. Top 81 is attached using straps 82 to the living mammal 86 (human being). When electrode wires 26A and 26B are attached to power supply 83 via lines 84 and 84A a circuit is completed and sampling of the substanc is collected in el ctrically conducting gel 25A or 25B. Th main difference betw n top half 11 and top 81 is that th glass walls 15A and 15B, tc. extend to form a good s al on the horizontal membrane substrat at contact surface 85. (Power supply 83 may be small-watch size and portabl).

It is known that drugs or their metabolites, such as alcohol, aminopyrine, methylurea, acetamid , sulfauanidine, sulfadiazine, theorphylline, and other low molecular weight nonelectrolytes ar s creted through the skin or mucous membrane in sweat, saliva or the like. Oth r compounds or their matablites which may be indicative of certain normal or diseas conditions such as ph nyalanine (phenylketonuria), sugar (diabetes) estriol (pregnancy) calcium (neoplasms) and copper (leukemia) for example, as well as normal and abnormal metabolites of other substances may be secreted in such fluids. Fluid collection is also used experimentally for determining biological requirements of various substances such as magnesium. If fluid samples are obtained and analyzed for these materials, the presence of such materials in the body can be detected. Such fluid collection therefore is useful in a wide variety of experimental, diagnostic, therapeutic and forensic medical purposes. While such fluids can be collected in numerous ways, the present invention describes a method of collection using a electrical current.

Additional compounds of interest are found for sampling or delivery to a human being in "Iontophoretic Devices for Drug Delivery," by Praveen Tyle, Pharmaceutical Research, Vol. 3, #6, pp. 318-326. Specific substances of interest for sampling as an ionic or a nonionic species is found on page 320 and is included below as Table 4..

Table 4

Substances of Interest for Delivery or Sampling

Drug	Condition/disease	Reference (No.)
1. Methylene blue and potassium iodide	Skin disorders (e.g., Demodex infection)	Jenkinson and Walton (7)[a]
2. Pencillin	Burns	Rapperport *et al.* (10)[a]
3. Histamine	Disease conditions of soft tissues, bursae, and tendons	Kling and Sashin (11)[a]
4. Sodium iodide	Electrolytes	Strohl *et al.* (15)[a]
5. Sulfa-drugs	Pyocyaneus infection	von Sallmann (16)[a]
6. Dexamethasone, sodium phosphate, xylocaine	Musculoskeletal inflammatory conditions	Harris (17)[a] Delacerda (18)[a]
7. Copper	Contraception	Riar *et al.* (19)[a]
8. Insulin	Diabetes	Karl (21)[a] Stephen *et al.* (22)[a]
9. Pilocarpine	Cystic fibrosis	Webster (23)[a]
10. Ragweed pollen extract	Hay fever	Abramson (31)[a]
11. Phosphorus		O'Malley and Oester (35)[a]
12. Water	Hyperhidrosis	Tapper (43)[a]
13. Citrate	Rheumatoid arthritis	Coyer (51)[a]
14. Dexamethasone Na phos. and lidocaine HCl	Primary tendonitis	Bertolucci (52)[b]
15. Hyaluronidase	Hemorrhages	Boone (53)[a]
16. Vidarabine monophosp. (Ara-AMP)	Keratitis (herpes virus)	Kwon *et al.* (54)[a] Hill *et al.* (56)[a]
17. Lignocaine HCl or lidocaine	Topical analgesia	Comeau *et al.* (9,28)[a] Russo *et al.* (12)[a] Echols *et al.* (27)[a] Siddiqui *et al.* (38)[a] Petelenz *et al.* (55)[a] Schleuning *et al.* (59)[a] Gangarosa (60,61)[a] Arvidsson *et al.* (62)[a]
18. Acetyl beta methylcholine Cl	Arteriosclerosis	Cohn and Benson (57)[a]
19. Acetyl beta methylcholine	Arthritis	Martin *et al.* (58)[a]
20. Idoxuridine	Herpes simplex, keratitis	Gangarosa *et al.* (60,63)[b]

Table 4 continued in next page

Table 4 (continued)

Substances of interest or Delivery or Sampling using Iontophoresis

21. Sodium floride	Dentin	Gangarosa (60)[a]
22. Methylprednisolone succinate	Postherpetic neuralgia	Gangarosa *et al.* (64)[a]
23. Lidocaine, epinephrine, and corticosteroid	Temporomandibular joint–myofascial pain dysfunction syndrome	Ganagrosa and Mahan (65)[a]
24. Sodium salicylate	Planter warts	Gordon and Weinstein (66)[a]
25. Calcium	Myopathy	Kahn (67)[a]
26. Acetic acid	Calcium deposits	Kahn (68)[a]
27. Zinc	Nasal disorders	Weir (69)[a]
28. Esterified glucocorticoids	Peyronie's disease	Rothfeld and Murray (70)[a]
29. Vasopressin	Lateral septal neuron activity	Marchand and Hagino (71)[c]
30. Alkaloids	Chronic pain	Caillik *et al.* (73)[a]
31. Optidase	Arthrosis	Ulrich (74)[a]
32. Natrium salicylicum butazolindin	Acute thrombophlebitis	Kostadinov *et al.* (75)[a]
33. Penicillin	Pneumonia and abscesses of lungs	Sokolov *et al.* (76)[d]
34. Papaverine and nicotinic acid	Cervical osteochondrosis with neurological symptoms	Ostrokhovich and Strelkova (77)[d]
35. Grasses	Allergy	Shilkret (80)[a]
36. 6-Hydroxydopamine	Ocular infection	Caudil *et al.* (81)[d]
37. Metoprolol	Beta-blocker (angina pectoris)	Okabe *et al.* (82)[d]

a Based on clinical impressions (qualitative).
b Based on double-blind study (well-controlled study).
c Based on *in vitro* experiments.
d Based on controlled comparative study (quantitative, but not double blind).

Delivery-In-Vivo

The same type of device as shown in Figures 7, 8, 9A, 9B and 10 for sampling in vivo can be used for the in vivo delivery.

Biosensing Using Iontophoresis

In this aspect Figures 5, 7, 8, 9A and 9B are of importance. The description abov for in vivo sampling is of interest plus an analyzing component.

The analyzing component may be (ion) specific electrodes, selective electrodes, electronic biosensors that connect specific biochemical changes to electric signals, colorimetric reagents and the lik .

The indication of the presenc of the substance of interest in the tissu of the patient may be qualitative or quantitative. Th measured value may be linked to a drug delivery unit to provide an additional level of a therapeutic agent.

The sampling of the component may be by a single sampling iontophoresis electrode.

The analytical method when it senses that the substance (or bioactive material) in question has changed may automatically administer an appropriate level of need therapeutic agent. The measurement may also simply alert an operator that therapeutic agent needs to be added orally, dermally, rectally, buccally, intravenously or the like.

Advantages of the In-Vivo or In-Vitro Iontophoretic Sampling or Delivery

1. The sampling approach outlined herein is a simple, convenient and painless technique for sampling bioactive materials with the purpose of diagnosis or monitoring. Sampling can be continuous or periodic.
2. The sampling is highly significant in situations where a routine blood sample could not be drawn, or where acquisition of multiple blood samples is undesirable (e.g. from an infant).
3. The sampling technique offers characteristics that may ultimately be engineered into a "biofeedback" loop-type system. In other words, the iontophoretic device, while permitting sampling by the method described, can also be used to deliver a therapeutic agent by any administration route (i.e., in response to a need "sensed" by the sampling).
4. The sampling will make outpatient monitoring safe and simple and provide a use of iontophoresis of wide and rather general applicability.
5. The sampling or delivery of any bioative material can be modified if the sampled biomaterial does not cross the skin sufficiently rapidly: agents (e.g. alcohols, fatty acids, glycols, Azone , etc.) which lower the local barrier function of skin can be incorporated into the electrode device to improve the extraction or delivery flux.
6. The technique is amenable to sampling under both electrodes, i.e. to the simultaneous determination of more than a single bioactive agent (e.g. a drug and a metabolite or conjugate of the drug).
7. The technique as indicated, would not be solely limited to biomaterial sensing across the skin. Other mucosal surfaces are also suitable for the approach. Examples include the nasal mucosa, the rectum, the vagina and the inside of the mouth. These surfaces are presently used as sampling sites with varying degrees of success. Since these mucosal surfaces are, in general, well perfused by small blood vessels, and because these membranes are less resistive to molecular transport, small current applied for shorter times can be used when sampling from these tissues.
8. The technique has an efficiency which depends upon the current applied between the electrodes and the duration of current flow. These variables can be precisely controlled enabling reproducible sampling and permitting, thereby, the generation of reliable data for comparative purposes (e.g. contrasting the levels of a particular bioactive material before, during and after a therapeutic treatment). This would be a conspicuous advantage when sampling from the nose, a notoriously difficult site from which to obtain reproducible information.
9. Bioactive materials or substances which are charged or uncharged are candidates for iontophoretic administration or sampling. These include small protein, di- to polypeptides, lipophobic and lipophilic materials. Charged materials which often cannot be administered easily by other routes are preferred. See substances in U.S. Patents numbers 3,731,683 and 3,797,494, which are incorporated herein by reference.
10. The device makes it possible to sample or administer in vivo or in vitro a substances (or bioactive material) wherein the electrodes are on the same side of the subject surface. The sample is generally in a horizontal confirguration while the electrode materials are adjacent to each other and in a generally vertical orientation. If the top of the device is sealed, it can be at any degree of orientation on the skin so long as electrical contact with the membrane surface is not impaired.
11. This device and technique makes it possible to sample or deliver drugs both systemically or locally. For instance, it is possible to use this technique to treat a skin cancer with methotrexate administered through the skin.

The following Examples are to be read as being illustrative and exemplary only. They are not to be construed as being limiting in any way.

IN VITRO SAMPLING

Testing of the Modeling Cell: Glass diffusion cells as described above (see Figure 1, 2, 3 or 4) were made by Skin Perm ation Systems (L.G.A., Berkeley, CA). The cell 10 is a modification of a standard flow-through diffusion cell (LGA skin penetration cell catalog no. LG 1084-MPC), described by Gummer et al., International Journal of Pharmacology, Vol. 40, p. 101 ff, published in 1987.

The top half of the cell is divided into three compartments or chambers (16A, 16B or 16C) by two walls 15A and 15B so that the only physical/electrical connection between the two electrode chambers (16A and 16C in Figure 1, 2, 3 or 4) decreases the possibility of leakage between them and makes it possible to investigate questions involving skin continuity. The top half of the cell 12 has a channel 18 or trough below this space that isolates the skin from the rest of the receptor phase. Filling this channel 18 with receptor fluid 19 during an experiment keeps the skin above it moist. The bottom half of the cell 12 also has ports 20A and 20B for the continuous flow of the receptor phase 17 and ports 21A and 21B for water jacket 21C circulation. Capillarity between the compartment walls and the external well was prevented by silanizing the top of the cell with dichlorodimethyl silane (Aldrich Chemical Co., Milwaukee, WI). The cells used with a three-station magnetic stirrer unit and stirring bar 27. (LG-1083-MS, LGA, Berkeley).

Metal Electrodes Wires (26A and 26B) - Platinum wire (Pt wire - Fisher # B-766-5A, 99.95% pure).

Power Supply (291)-- Current or voltage control with automatic crossover (Model APH 1000M, Kepco, Inc., Flushing, NY). This supply has a specified drift of ≤2μA/8 hrs for its current-controlled output, an important consideration if drug flux is sensitive to changes in current.

Receptor Fluid (17) - Phosphate buffered saline (pH = 7.4, 0.9% NaCl W/V).

Dye - Blue dye #1 FD&C in deionized water.

Drugs - Clonidine HCl (Sigma Chemical Co., St. Louis, MO); Clonidine-HCl (phenyl-4-^{3}H) of specific activity 90 mCi/mg (Amersham, Arlington Heights, IL). Morphine sulfate (Sigma Chemical Co., St. Louis, MO); Morphine (N-methyl-^{3}H) of specific activity 255 mCi/mg (New England Nuclear, Boston, MA). The non-labelled drugs were dissolved in deionized water to form solutions of 1 mg/ml, with enough labelled drug to achieve an activity of approximately 1μCi/ml.

Skin (13) - Full-thickness skin, freshly excised from 11-15 week old female hairless mice (strain Skh:HR-1, Simonsen Laboratory, Gilroy, CA).

Testing of the Modeling Cell - The diffusion cell 10 was tested in three ways:

(1) Leakage tests (without current) using dye and silicone rubber than skin; (2) leakage tests using dye and skin (without current); and (3) iontophoretic tests using the drug solutions and skin (with and without current). Procedures (1) and (2) were evaluated by visually inspecting the cell. For procedure (3), 0.6 ml. of labelled drug solution was placed in chamber 16A, 0.6 ml of buffered saline was pipetted into chamber 16C, and a constant current of 0.63 mA/cm^2 (with the voltage limited at 9 V) was imposed between the electrodes in the two chambers. The activity of the solutions in chambers 16A and 16C was determined before and after iontophoresis. The activity of the skin 13 and of the samples taken from the receptor chamber was determined post-experimentally. Each experiment lasts approximately 24 hours ± 2 hours, with samples collected hourly. Receptor fluid 17 was magnetically stirred (27), and the collection flow rate was 10 ml/hr. Each procedure was repeated three times.

RESULTS

Procedures (1) and (2): No dye leakage was observed from the side chambers 16A and 16C to the middle chamber 16B, or from any chamber to the outside of the cell, for both the model silicone rubber membrane and the hairless mouse skin.

Procedure (3): When the dye in chamber 16A was replaced with a labelled drug and no current was applied, the drugs diffused into the receptor phase 17 with mean rates of 0.05 μg/cm^2/hr clonidine-HCl and 0.04 μg/cm^2/hr for morphine sulfate. In both cases, no drug was found in the buffered saline of chamber 16C after 20 hours.

When current was applied between the chamber with the labelled drug and the chamber with the buffered saline, permeation increased substantially. The rate of penetration of morphine sulfate through the skin with a current of 0.63 mA/cm^2 was 2.0 μg/cm^2/hr compared to the passive transport rate of 0.04 μg/cm^2/hr. In the case of clonidin -HCl, the rate changed from 0.05 μg/cm^2/hr without curr nt to 15.0 μg/cm^2/hr with an el ctrical driving force. Labelled drug was detected in the buffered saline of chamber 16C, with 1 μg of morphine sulfate present approximately 20 hours (see Figure 14), and 5μg of clonidine after the sam tim .

The labelled drug might have entered chamber 16C by s veral paths, the most likely being that it was "pulled" back up through th skin und r the passive electrode. To test this possibility, two c lls were connected by tub 95 so that their receptor phases 92 and 93 wer common but such that th skin and cell tops were physically separated (see Figure 10). Labelled drug and the positive electrode were positioned in chamber 91A of cell 90A. The negative electrode was placed into chamber 91B in cell 90B. All other chambers (91C, 91D, 92 and 93) were filled with buffered saline. The remaining experimental procedures (electrical parameters, sampling were identical to those of Procedure 3 above). Labelled drug was transported into chamber 91A of cell 90A when the cells were connected in this fashion, demonstrating the existence of a "reverse transdermal" path in iontophoresis, which is in fact sampling by iontophoresis.

(b) Additional materials which are expected to be sampled in a similar manner as described hereinabove for clonidine include for example, morphine, heroin, insulin, neomycin, nitrofurasone, and beta-methasone.

EXAMPLE 2

IONTOPHORETIC SAMPLING IN VITRO

Iontophoretic sampling consists of pulling out chemical substances from the body through the skin by means of electricity. In order to test this method, an iontophoretic in-vitro cell is used (Figures 1, 2, 3 or 4). Full-thickness hairless mouse skin 13 (8-13 weeks old) is placed between the two parts of the cell. Solutions of radiolabelled drugs of known concentration in phosphate-buffered saline are circulated 10 ml/hr. beneath the skin. On top of the skin there are two self-adhesive gel electrodes, connected to a power supply which is set up in constant current mode (0. 5mA). Current is applied for measured length of time (about 2 hr.) corresponding to about 0.63 mA/cm^2. After the experiment the gel electrodes are taken for scintillation counting, which reflects the amount of drug absorbed by the gel electrodes. Using different drug concentrations with the same electrical current for the same period of time, a linear correlation between the amount is collected by the electrode and drug concentration is expected.

Results of the above described method using clonidine and theophylline in various concentrations are given in the graphs (Fig. 11 and 12), each data point is a mean of at least two experiments. The graphic presentation of the data shows the linearity of the results.

IN-VITRO DELIVERY

(a) Morphine - The procedures and conditions described for in vitro sampling were used for delivery of morphine: Current 0.63 mA/cm^2 for 20 hrs. using the cell described in Figures 1, 2, 3 or 4. The morphine was placed in Chamber 16A. After iontophoresis, morphine was found in chamber 16C. (See Figure 14).

The electrically conducting gel for all experiments was KENZ-GEL-ELC gel of Nitto Electric Industrial Electric Company, Osaka, Japan.

(b) P.S.O.S. (Potassium Sucrose Octa Sulfate) P.S.O.S., 0.6 ml of 1.5 mg/ml solution in a boric buffer is placed in chamber 16A and boric acid buffer alone is placed in chamber 16C of Figure 1, 2 or 3. Using a direct current of 0.5 mA (0.65 mA/cm^2) transport of P.S.O.S. is observed up to 5 µg/hr. Using the same experimental configuration with a current only a few nanograms of P.S.O.S. was transferred. See Figure 15.

IN-VIVO SAMPLING

The procedure and description above for in vitro sampling is used except that the membrane is replaced by the top of the forearm of a 29 year old male human being. The sampling cell used is the one in Figures 7, 8, 9A and 9B. The amount of clonidine sampled is comparable to that observed in the in vitro case.

IN-VIVO DELIVERY

Figure 13 shows a diagram of a guinea pig having separate patch electrod s for sampling of bioactiv materials from th mammal.

In order to examine th invention in vivo one test has been conduct d so far. An iontophoretic procedure is applied on hairless guinea pig 133. On gel el ctrod 132(+) on lin 131 with theophylline (5.5 µg, 2.4 x 4.2 cm) is placed on one sid of th animal's back and another g l electrode 132A(-) on line

131A is attached t the other sid of the back (separation distance approx. 7cm). After 20 minutes at 1.0mA (power supply 130), the gel lectrodes ar removed and 1.83 ng of theophylline is found in the negative lectrod . The amount that is absorbed by th body of the guinea pig is 1.9 µg.

BIOSENSING USING IONTOPHORESIS

The sampling of the radioactive clonidine in vitro is described above. When that procedure is adapted for in vivo sampling in a dog, the level of radioactive clonidine is measured iontophoretically, quickly and accurately. The operator is alerted when to administer an injection clondine to maintain the desired level of clonidine in the test dog.

While only a few embodiments of the invention have been shown and described herein, it will become apparent to those skilled in the art that various modifications and changes can be made in the iontophoresis diffusion cell for in-vitro and in-vivo sampling of bioactive molecules and for the in-vitro and in-vivo delivery of bioactive molecules or in biosensing applications without departing from the spirit and scope of the present invention.

Claims

1. A method of substantially continuously monitoring the level of a bioactive material transferred through skin or mucosal membrane, the method comprising the following steps
   - placing separate electrodes on the skin or mucosal membrane;
   - applying current to the skin or mucosal membrane;
   - substantially continuously transferring the bioactive material through the skin or mucosal membrane and colleting the same; and
   - monitoring the collected bioactive material.

2. The method of claim 1 wherein the bioactive material is glucose or metabolic glucose.

3. The method of claim 1 wherein a biosensor is used to monitor the bioactive material in the monitoring step.

4. The method of claim 1 wherein the electrode is a gel electrode.

5. The method of claim 1 further comprising the step of providing feedback.

6. The method of claim 5 characterized in that the step of providing feedback comprise alerting an operator.

7. The method of claim 1 performed in vivo.

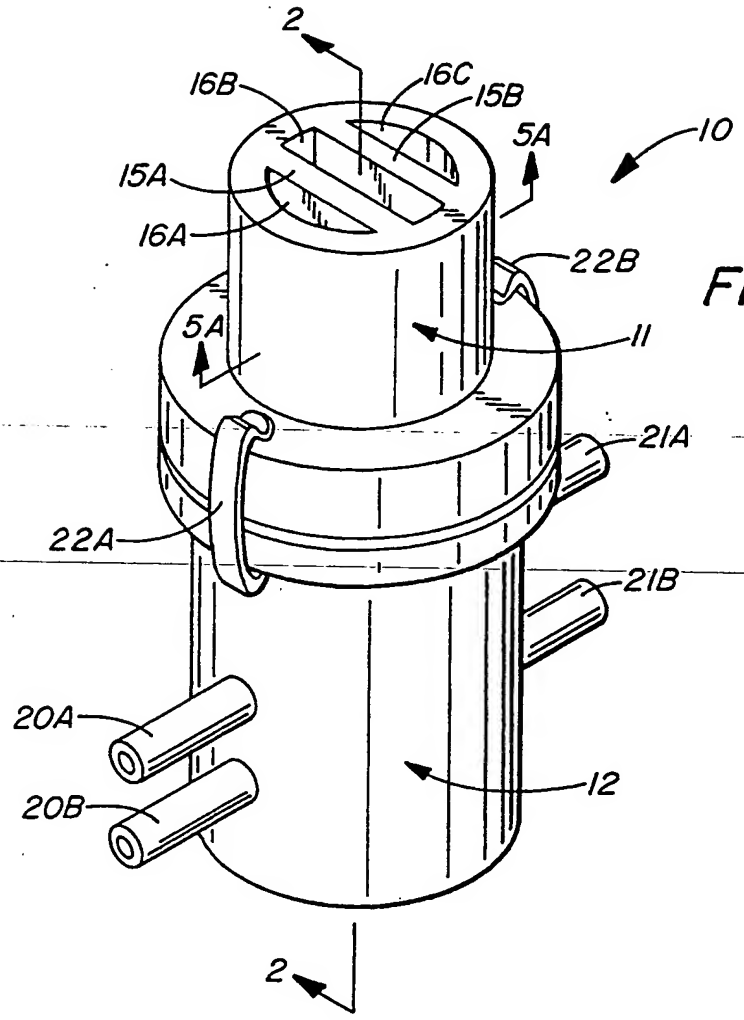


FIG._1.

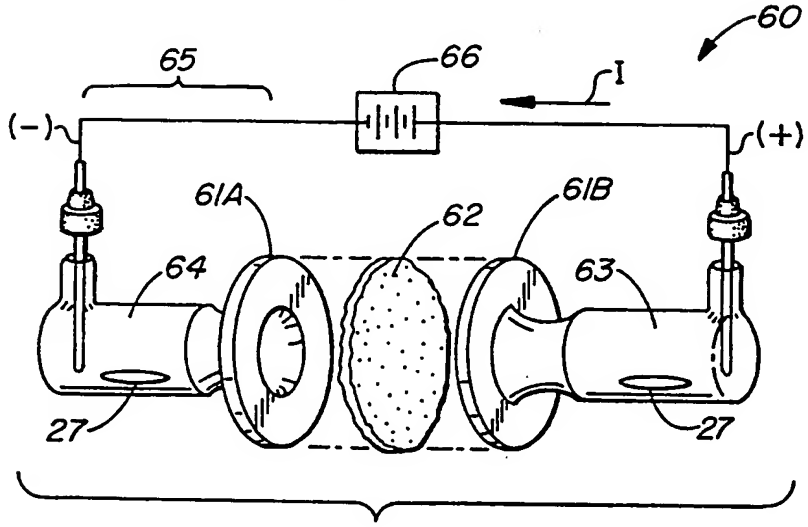


FIG._6. (PRIOR ART)

FIG._2.

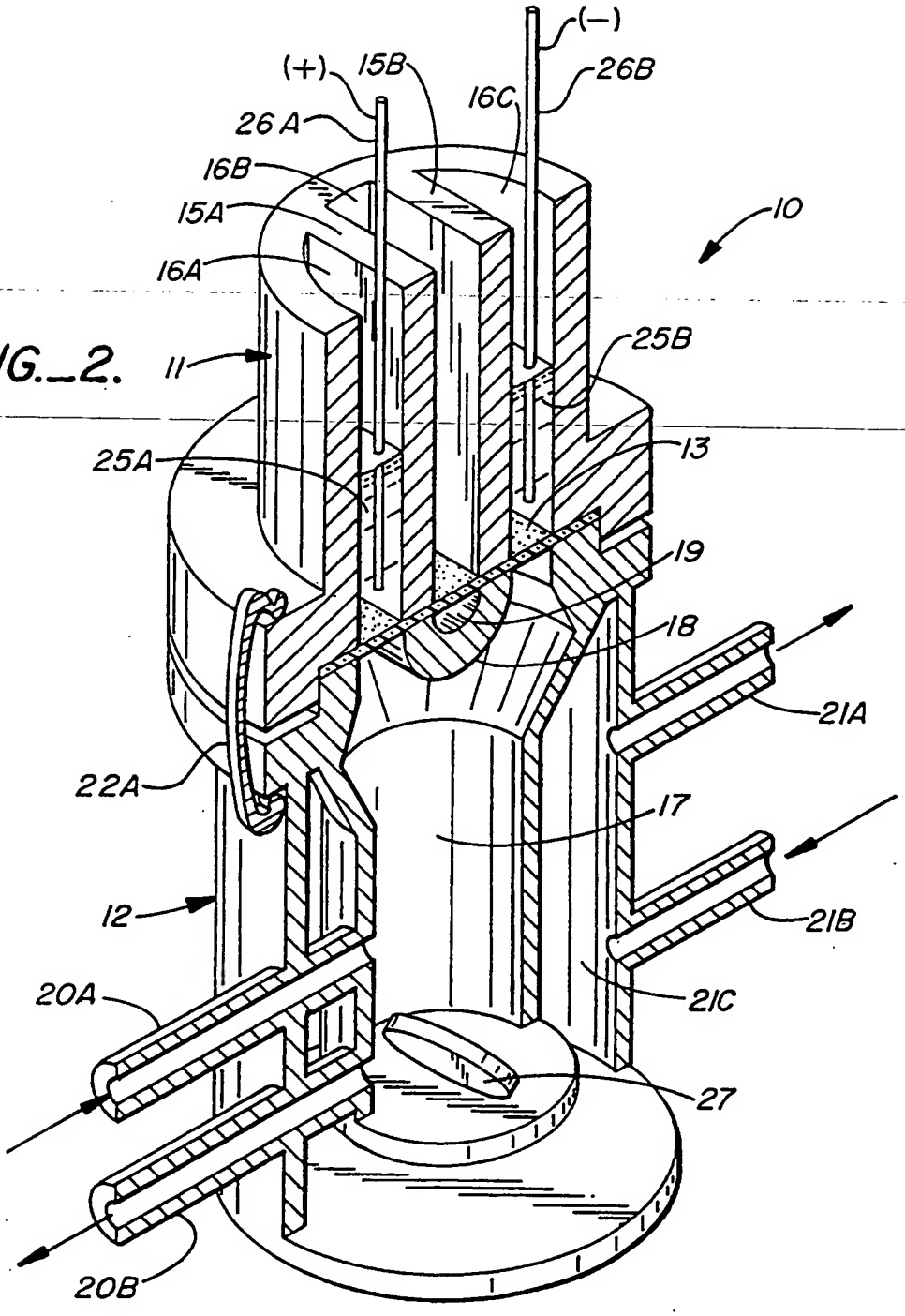

(+)
15B
(−)
26B
16C
26 A
16B
15A
10
16A
11
25B
25A
13
19
18
21A
22A
17
12
21B
20A
21C
27
20B

FIG._3.

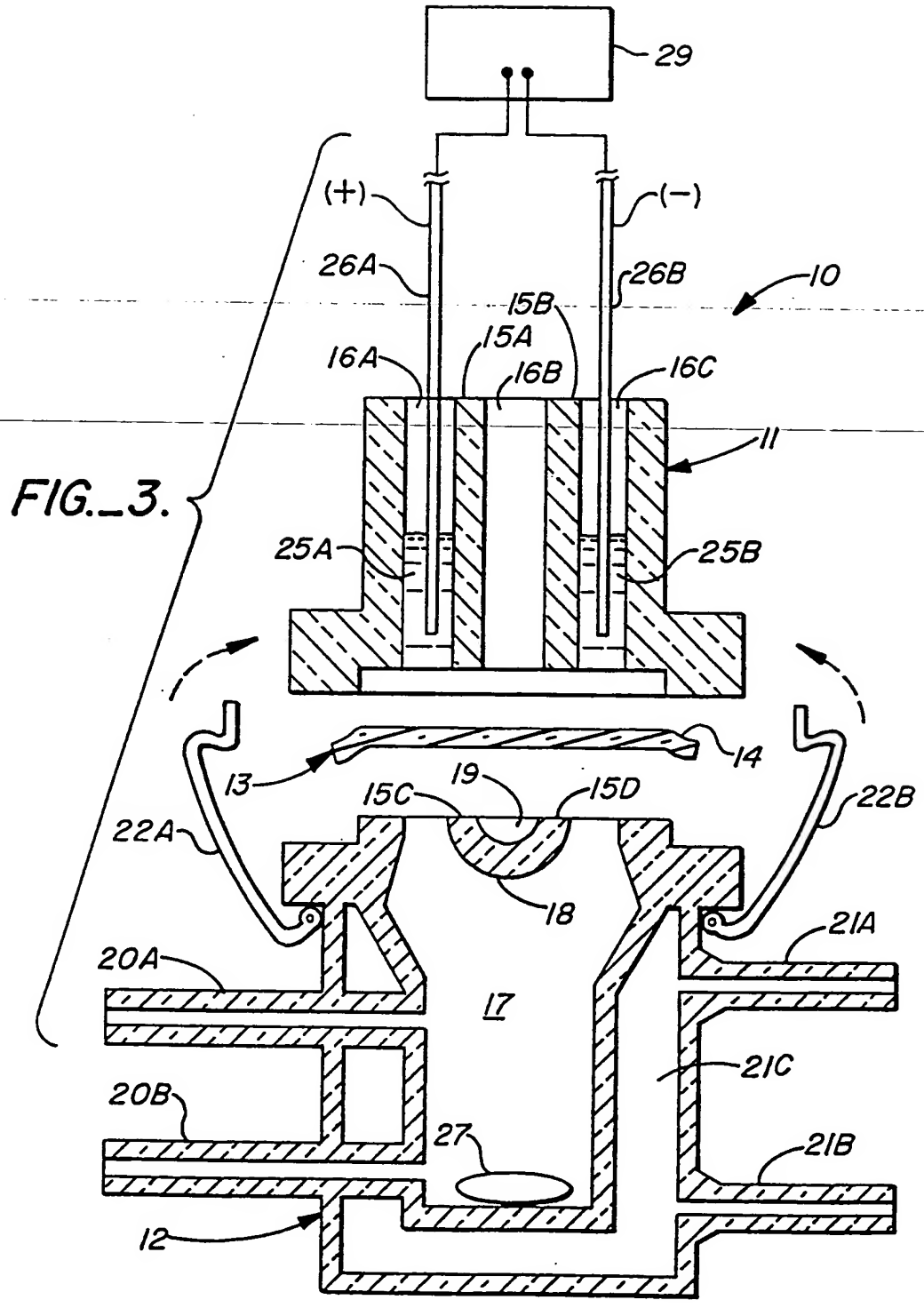
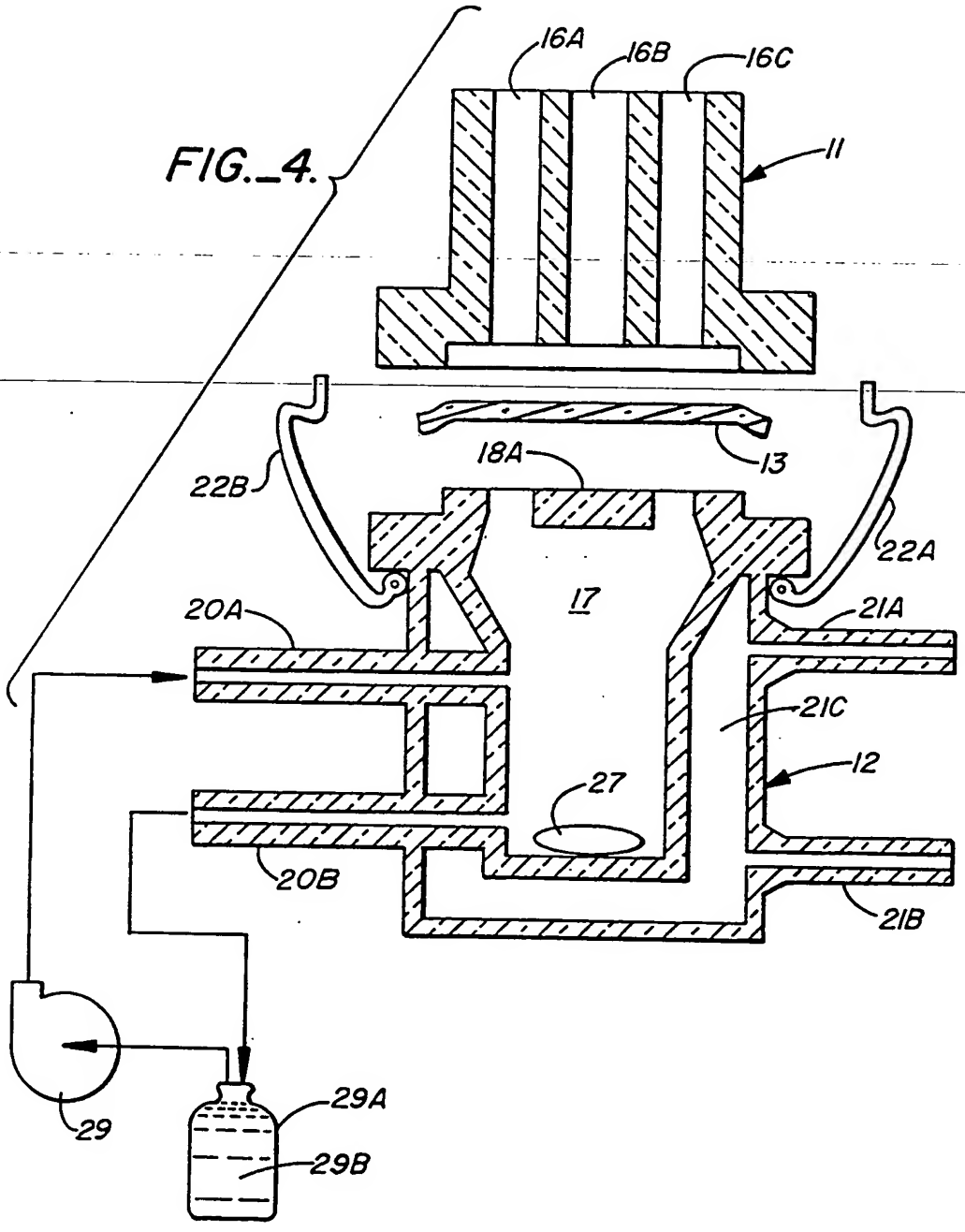

29
(+)
(−)
26A
26B
10
15B
15A
16A
16B
16C
11
25A
25B
14
13
19
15C
15D
22B
22A
18
21A
20A
17
21C
20B
27
21B
12

FIG._4.


16A
16B
16C
11
22B
18A
13
22A
17
20A
21A
21C
12
27
20B
21B
29
29A
29B

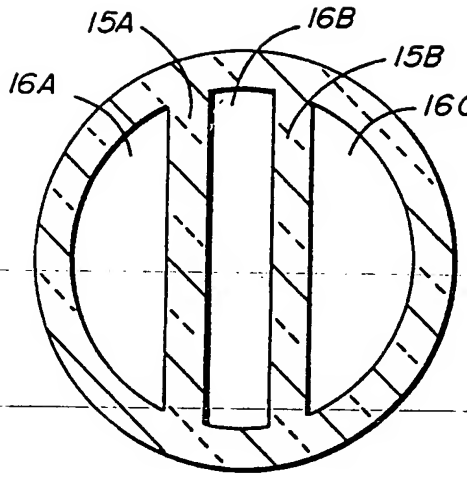


FIG._5A

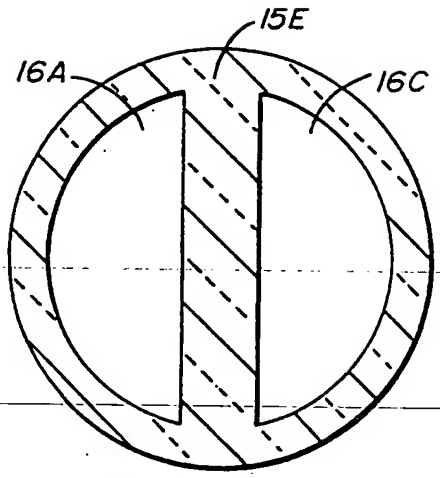


FIG._5B

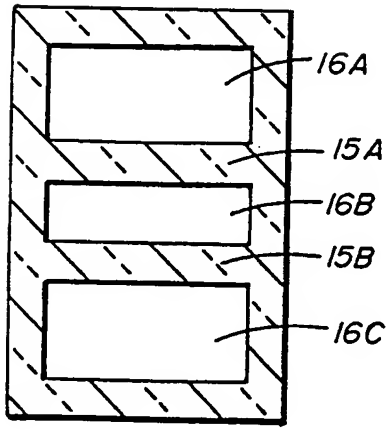


FIG._5C

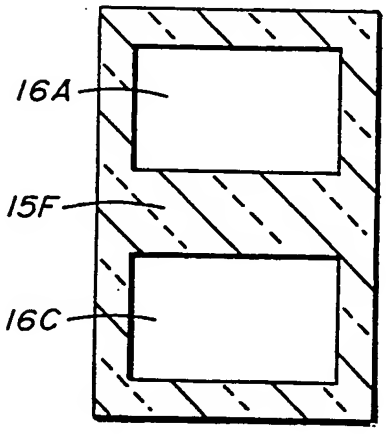


FIG._5D

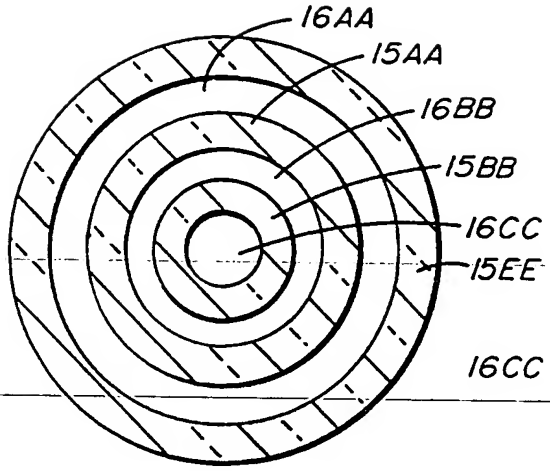


FIG._5E

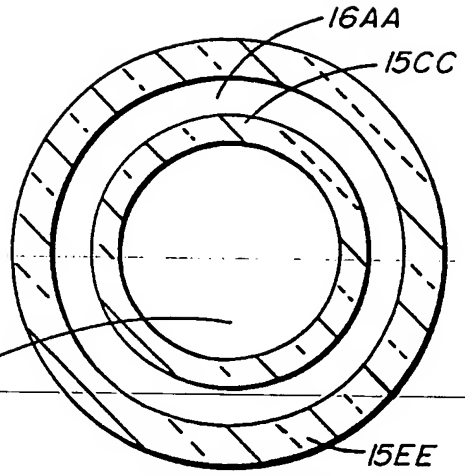


FIG._5F

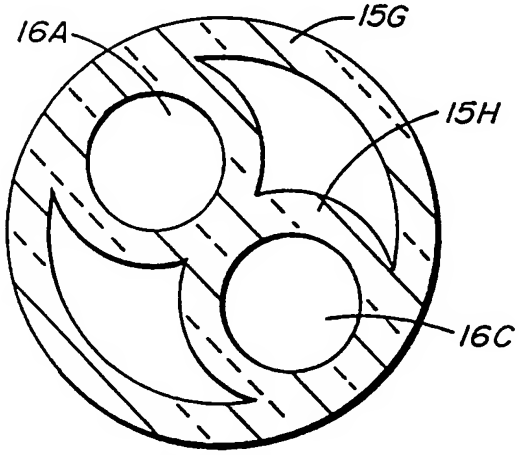


FIG._5G

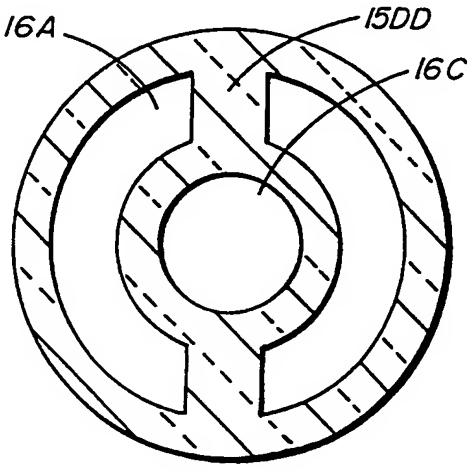


FIG._5H

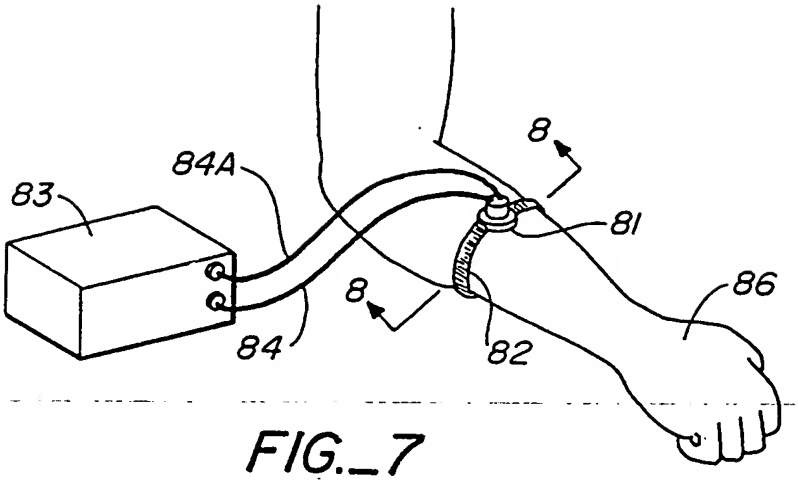

8
84A
83
81
86
8
84
82

FIG._7

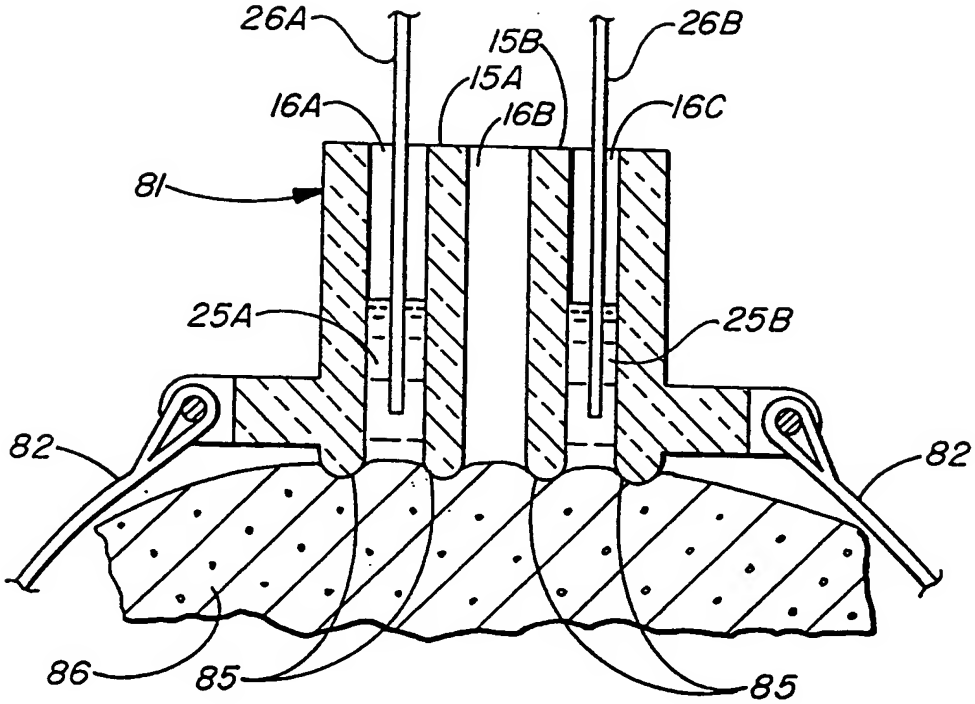

26A
15B
26B
15A
16A
16B
16C
81
25A
25B
82
82
86
85
85

FIG._8

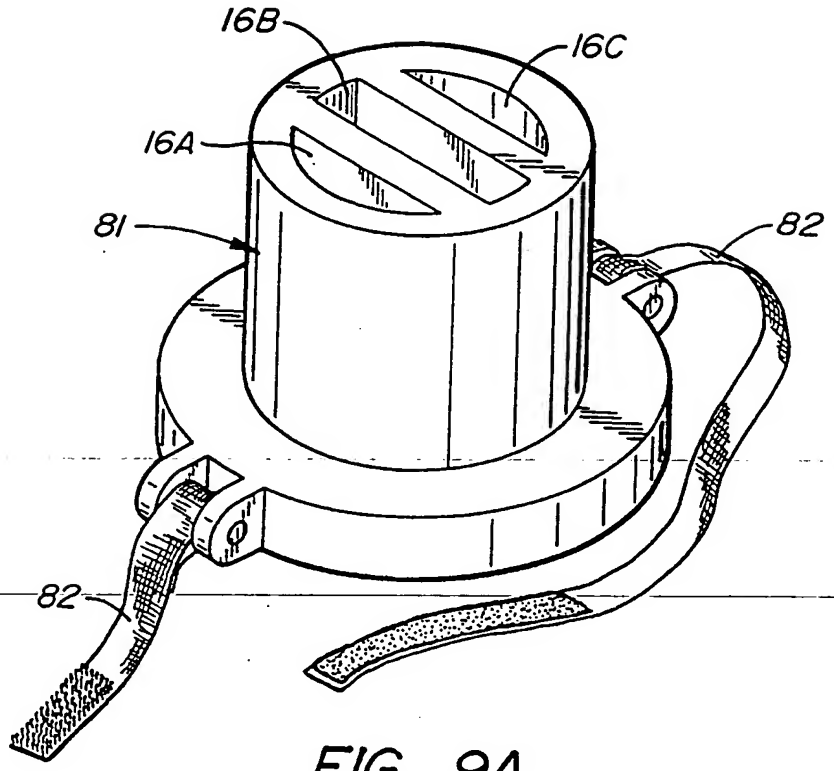


FIG._9A

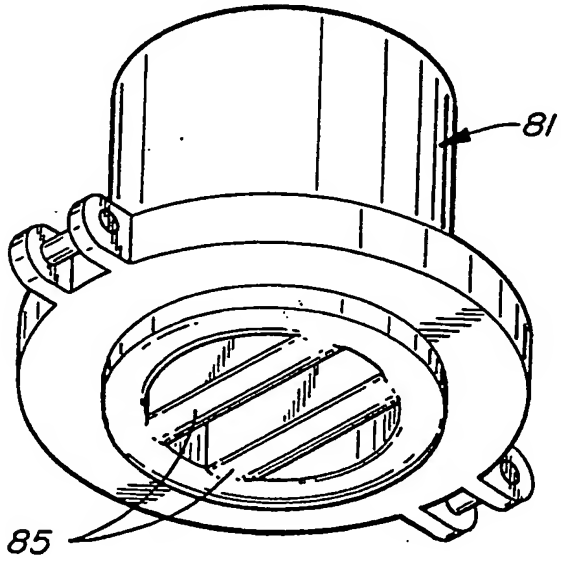


FIG._9B

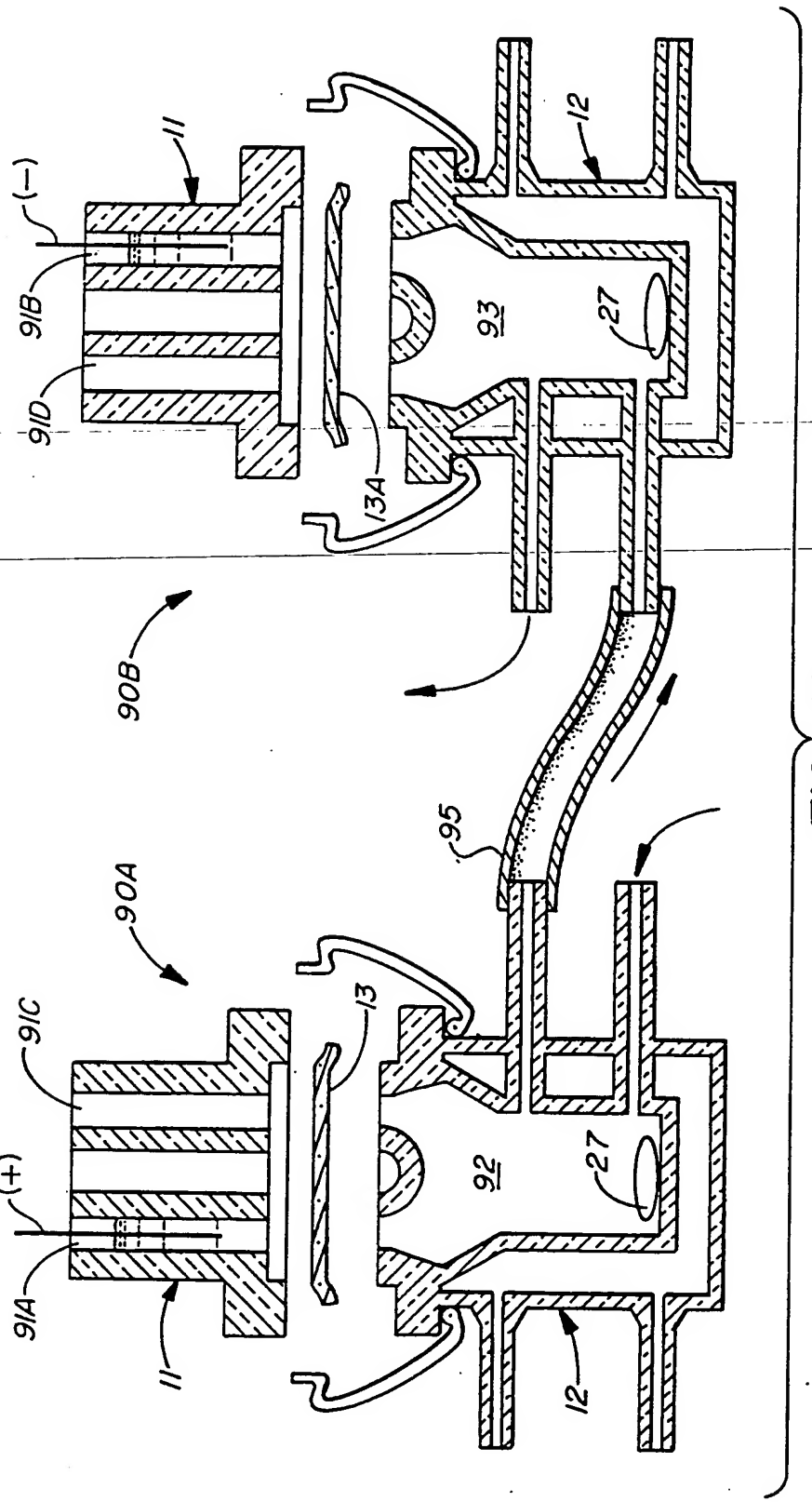


FIG._10.

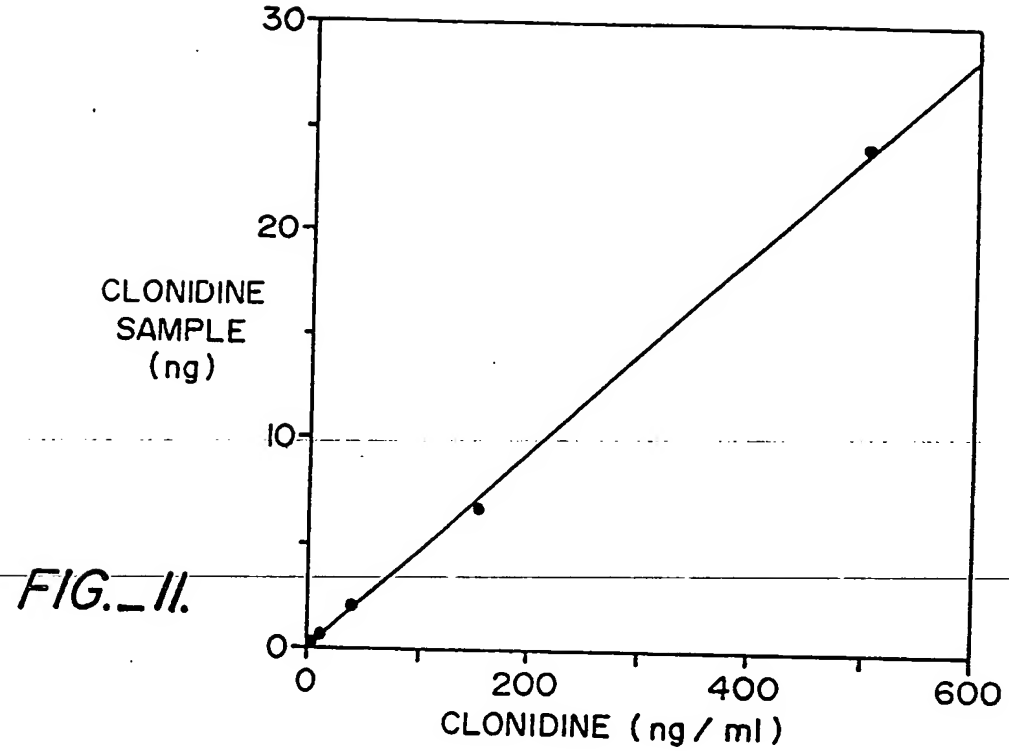

30
20
10
0
CLONIDINE
SAMPLE
(ng)
0
200
400
600
CLONIDINE (ng / ml)

FIG._11.

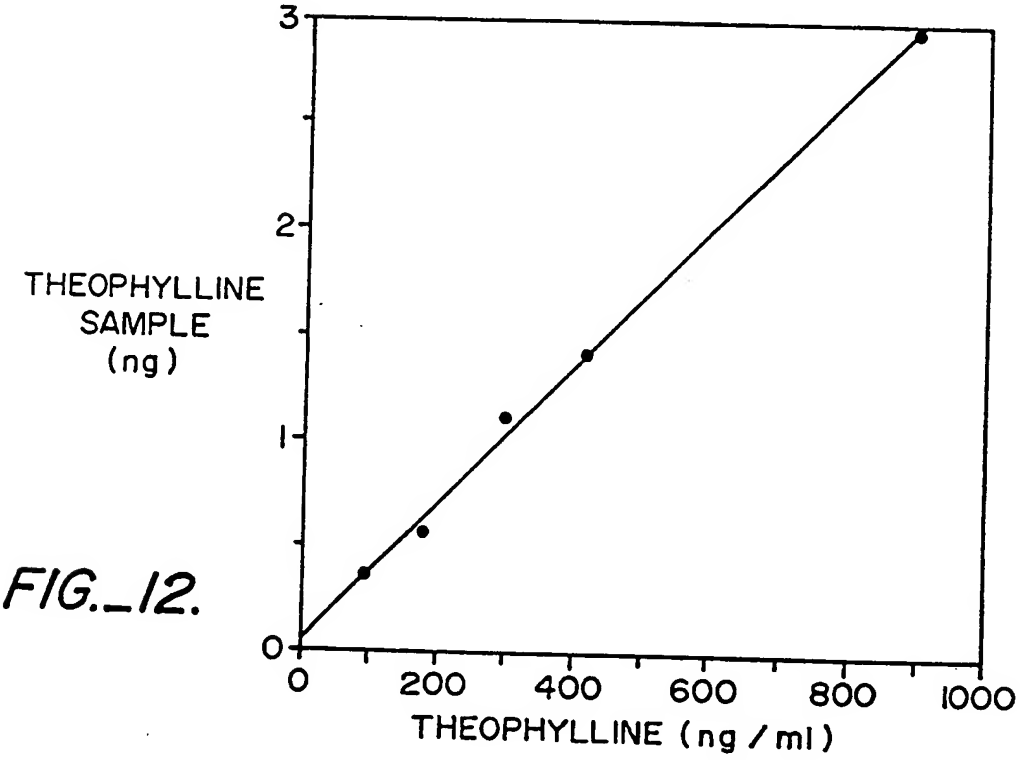

3
2
1
0
THEOPHYLLINE
SAMPLE
(ng)
0
200
400
600
800
1000
THEOPHYLLINE (ng / ml)

FIG._12.

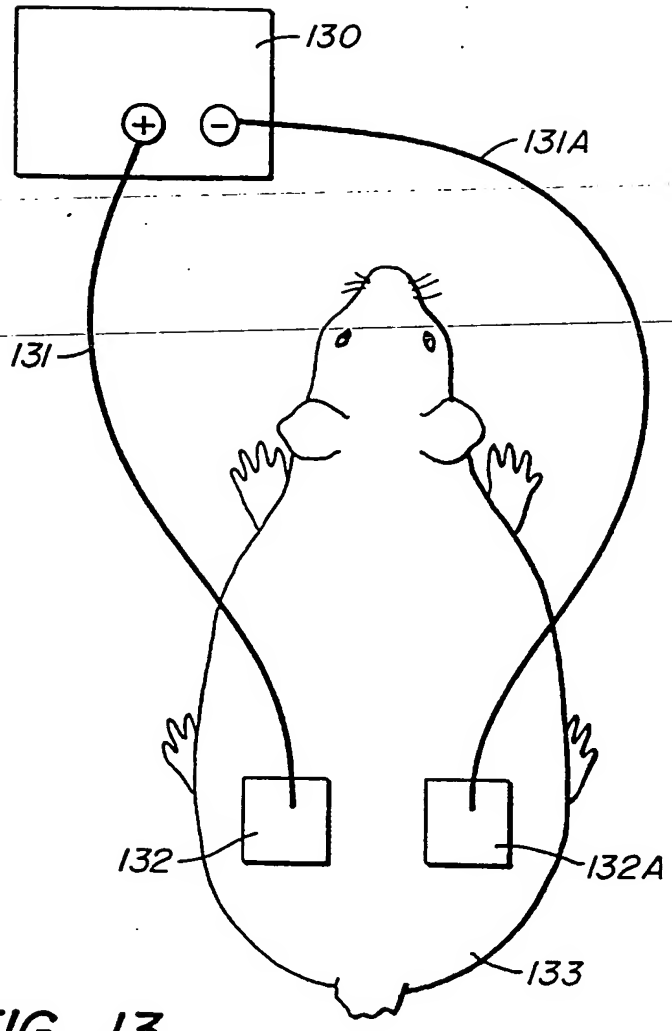


FIG._13.